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UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY

FOREST INSECT INVESTIGATIONS

TEMPERATURE REQUIREMENTS FOR PUPATION OF THE WESTERN PINE AND MOUNTAIN PINE BEETLES

By

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A Cooperative Study Conducted by
the University of California and the
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INFORMATION CONCERNING THE TEMPERATURE REQUIREMENTS FOR PUPATION OF THE WESTERN PINE AND MOUNTAIN PINE BEETLES

REPORT OF LABORATORY STUDIES--SEPTEMBER, 1934-May, 1935

INTRODUCTION

Pupae of the Western Pine Beetle (Dendroctonus brevicomis Lec.) are rarely, if ever, found in their natural habitat- the outer bark of ponderosa pine- during the winter period. With the approach of winter conditions in the field, pupae, which have formed during the fall, will transform to new adults. However, mature larvae in the same broods will remain dormant until the following spring. Why do these larvae go into the overwintering condition in this stage rather than transform to pupae, the usual resting stage for insects? It is apparent that some factor prevents the change to the pupal stage which, at the same time, permits the transformation of pupae into adults.

From the data about to be presented, although incomplete, three rather significant facts will be pointed out which may partially answer this question. These are as follows:

(1) It has been tentatively demonstrated that, in the fall of the year, the physiological change from the last active, 4th instar larval stage, to the prepupal condition acts as a bar or a barrier and prohibits the transformation to the pupal stage at temperatures near the zero of development (about 50° F.).

(2) Once the prepupal condition has actually been attained, the metamorphosis to the pupal and thence to the callow adult stage will continue uninterrupted at temperatures as low as 45 degrees F. This is 5 degrees F. below the previously suggested zero development, even though a minimum of 86 days is required for this change at this temperature. Indications are that this phenomenon will take place at temperatures as low as 41 degrees F.

(3) For larvae in the overwintering condition, although apparently mature, an additional period of feeding or preparation appears to be necessary during the winter, which enables them to transform readily to pupae and thence to adults with the advent of warmer weather in the spring. Subjected to a constant temperature of 50 degrees F.- which has been taken as the zero of development- overwintering mature larvae in the early fall (October) required a minimum of 156 days before pupation began; while similar larvae in the early spring (March), treated in exactly the same manner, required 21 days before pupation began.

The fact that pupae of the Mountain pine beetle (Dendroctonus monticolae Hopk.) have been found during the winter in their natural habitat - the cambium layer of sugar pine- indicates that the temperature requirements for pupation differ from those of the Western pine beetle.

While the experiments with the Mountain pine beetle, included herewith, have not been as successful as those with the above species, three conclusions may be drawn.

(1) The transformation from the active mature larval stage to the prepupal stage has not occurred at temperatures below 50° F. This indicates that the zero of development for mature larvae is very close to that of the Western pine beetle.

(2) It appears that it is the physiological change from the active mature larval stage to the prepupal condition that prohibits the change from mature larvae to pupae at temperatures below 50° F.

(3) After reaching this prepupal condition, development to the pupal and callow adult stages will take place at temperatures as low as 36° F. This probably accounts for the fact that pupae are found during the winter.

These conclusions have been warranted as a result of a research project conducted on a part time basis and made possible by a special fund of the College of Agriculture of the University of California. The work has been directly supervised by Mr. J. M. Miller and Dr. K. A. Salman of the U. S. Bureau of Entomology and Plant Quarantine.

Acknowledgement is hereby made and appreciation extended to Professor W. B. Herms for making possible the project; to Mr. Miller and Dr. Salman for invaluable constructive criticisms and suggestions; to Dr. E. C. VanDyke for helpful suggestions; to Mr. E. E. Patterson for the photographic detail, and to Mr. J. S. Yuill for help in the wiring of the constant temperature cabinets.

PURPOSE OF PROJECT

This study was initiated with a three-fold purpose:

(1) To determine the temperature requirements for pupation of the Western pine beetle (Dendroctonus brevicomis Lec.) and the Mountain pine beetle (Dendroctonus monticolae Hopk.)
(2) To provide a project which would be of aid to the Department of Entomology of the University of California in the teaching of research methods and problems in Forest Entomology.
(3) To add to the accumulating knowledge concerning the life histories of these primary barkbeetles by establishing a project which could be completed during the school year.

WESTERN PINE BEETLE STUDIES
Dendroctonus brevicomis, Lec.

Review of Previous Work

Because of the divergent behavior and requirements of these two species, each will be discussed separately.

The several studies already made on the relation of temperature to the Western pine beetle have concerned themselves chiefly with its lethal effects. However, Beal (1) and Smith (2) have dealt with temperatures affecting brood development.

Beal subjected the various brood stages to constent and varying temperatures and found that development begins at about 50 degrees F., and the rate increases directly with the temperature to about 85 degrees F.

Smith's results were a little inconsistent. After exposures of 54 to 60 days at constant temperatures of 40, 36, and 32 degrees F., some indications of development were noted. Smith concluded that those insects near the change from larva to pupa and pupa to adult did, in some cases, complete the change under these conditions.

Equipment and Methods Used in This Study

Temperature:

Throughout this experiment, the material was subjected to constant temperatures. Beal (1) has pointed out that alternating temperatures appear to have little stimulating effect upon brood development, but that the application of effective temperatures influenced development.

Space was available in controlled, constant temperature rooms belonging to the University in the basement of Hilgard Hall. Here, constant conditions of 45, 41, 36, and 32 degrees F. were obtained. In the laboratory of the Bureau in Giannini Hall were 4 ply-wood cabinets. One of these was equipped with a constantly burning light, in series with a small fan to insure air circulation, which maintained a fairly constant temperature of 74 degrees F.

In order to obtain controlled conditions between 45 and 74 degrees F., it was necessary to construct and equip units which would give the desired variation. There were 4 portable, cubical, cabinets constructed and patterned after some belonging to the University. (Plate No. 1). These were made of double thickness of 7/16 inch Masonite over a wooden frame and with a 1/2 inch air space between the inner and outer walls. The heat was furnished by lamp globes of various wattage. One burned constantly and another was on an intermittent circuit connected with a Mercury "U" tube thermo-regulator and a relay switch. A small 6 inch fan was installed to prevent air stratification. With this easily adjusted apparatus very satisfactory control was obtained. As long as the mercury in the "U" tube was kept reasonably clean, the variation was less than a half degree from the desired temperature. These cabinets were placed in the basement of Hilgard Hall with an outer air temperature of 36° F. and adjusted to give inner temperatures of 65, 60, 55, and 50 degrees F. (Plate No.2)

Humidity:

The problem of the standardization of relative humidity in these cabinets was a bit more difficult. During the previous tests with larvae, either in their natural habitat of outer bark or exposed in various containers, humidity has remained uncontrolled, yet it was recognized that its effects may have had some bearing on the results obtained.

In order to arrive at a reasonable yet accurate point at which to stabilize the relative humidity for this study, 12 samples of outer bark from #3B, 3C, 3D, 4B, and 4C trees (Keen's classification for Pinus ponderosa Lawson) were collected by Dr. Salman on October 10, 1934. These samples were placed in small tin cans and brought into the laboratory. Here they were weighed on an analytical balance and dried in an electric oven at 100-110 degrees F., until a constant weight had been reached. The average moisture content of these oven-dry samples was 12%.

Reference was then made to the correlation, obtained by Hatfield (5), between the equilibrium moisture content of oven-dry Southern yellow pine and the known relative humidities produced by supersaturated salt solutions at constant temperatures. It was found that Southern yellow pine when in equilibrium with a solution of $Na\ Br.2\ H_2O$ (which at 20 degrees C gives a relative humidity of 58%) has a moisture content of 12.4%. Then from the International Critical Tables (4) a cheaper salt - Calcium Nitrate-was chosen, which would give a relative atmospheric humidity of 56% at 18.5 degrees C (65.3 F). When the bark cells used in this study were in equilibrium with supersaturated solutions of this salt, conditions should be comparable to the previously mentioned 12% moisture content.

Solutions of this salt were placed in open dishes in the smaller cabinets. A closed battery jar containing this salt, over which larvae could be suspended, was placed in the 45 degree room.

While, as it has been pointed out by Sweetman (5), the reported relative humidities that can be obtained from various salt solutions cannot be accepted without being checked, the difficulties of measuring humidity in these small cabinets was not overcome. An "egg beater type" psychrometer was used at intervals to obtain the conditions inside of the box. But, since the measurements were taken with the door open, the results were only indicative and not positive. The average humidities remained between 55 and 65%. In the larger rooms, where humidities could be measured with a sling psychrometer, results of 50 - 60% were obtained.

Containers:

The difficulties of working with larvae in samples of outer bark have been pointed out on previous occasions. In order to segregate these larvae and deal with only one brood stage, various types of containers were tried which would simulate natural conditions and yet permit observation without undue disturbance.

(1) Squares of Ponderosa Pine bark 1-1/2 to 2 inches thick were sawed thru the middle. In one half 50 or 100 shallow holes were drilled with a 1/4 inch drill. The other half served as a cover and was held tightly in place with strong rubber bands. This type container rendered complete isolation and a natural medium after the larvae were removed from their original galleries in the outer bark. (Plate No. 3.)

(2) Later, with a shortage of thick bark, these sections were sawed into plates about 1/2 inch thick and in which similar holes were drilled. These were wrapped in cellophane and black paper held tightly in place with rubber bands. The ends were left exposed to allow the plates to come to equilibrium with the surrounding air, although the larvae were protected. After the first trial, this type container was used in all the other tests. (Plate No. 4.)

(3) An attempt was made to follow the development of second and third instar larvae and those of the fourth that had not completed their feeding, by using a method suggested by Dr. Salman. These larvae were placed in holes 1/16 to 1/8 inch in diameter drilled in sections of outer bark which had been sanded to a thickness of 1 to 1-1/2 millimeters. These sections holding 25 larvae were placed between glass plates and fastened with rubber bands. This method worked quite well for those larvae that were more nearly mature. They would enlarge their original round cell to an oblong one and, after feeding for a short period, pupated. With the smaller larvae it was difficult to place them in the proper size cells. A few did extend their cell 1/2 to 3/4 of an inch, pupate, and emerge as small adults. (Plate No. 5)

Relation of Time and Temperature to Pupation

At the beginning of this experiment the temperature of 50 degrees F., as suggested by Beal (1), was accepted as the practical zero of development, i.e that temperature below which successful development ceases and above which its rate increases directly with the rise in temperature. However, as these experiments progressed, evidence pointed to a zero of development which varied with:

(1) The stage of the brood - whether larva, prepupa, or pupa, and

(2) The period of the winter during which the larvae were removed from the bark.

This discussion of the relation of time and temperature to pupation will be discussed under two divisions.

A. Effect upon larvae removed from the same bark samples and subjected to different constant temperatures.

B. Effect upon larvae removed from bark collected at intervals during the winter and subjected to the same constant temperatures.

A. The Effect of Time and Temperature upon Larvae removed from the Same Bark Samples and subjected to d different Constant Temperatures:

Test No. 1- Started November 10,1934

Object: To make a general test for the zero of development and to determine the temperature requirements for pupation.

Bark: Ponderosa pine from Lassen N.F., collected Oct.25,1934, by Boyce berg.

Container: Thick bark plate with thick bark cover

History: After collection, the bark was placed on the Oxford St. lot in the shade. Larvae removed Nov. 9, 1934, and placed directly into bark cells at lower temperatures.

Summary of Results: TABLE NO. 1

Temperature: Degrees F.	Percent Mortality	Number of Days after the Start of the Test: Pupae: First	Pupae: Last	Callow Adults: First	Callow Adults: Last
74	35	7	18	14	24
65	30	16	43	24	55
60	40	22	51	40	81
55	44	14	130	42	167
50	*	156	*	198(?)	*
45	*	*	*	*	*

*= Not completed May 6, 1935

Discussion: This, the first attempt, served as a trial and error test and pointed to weaknesses in the method of handling larvae and in notekeeping and other points.

With the exception of the appearance of pupae at 55 degrees F. before any appeared at 60 degrees, these results were about as expected. That is, that as the temperature nears the zero of development the rate of development decreases. With no evidence of pupation at 50 degrees F. after four months' exposure, this problem appeared relatively simple and pointed to a temperature between 50 and 55 degrees F. at which activity ceased. However, after 156 days of continuous exposure and without any additional feeding, the appearance of the first pupae at 50 degrees F. changed the problem from a relatively simple to a complex one.

The first callow adult has not yet appeared at 50° F. At the time of writing this report, the pupae have begun to color indicating that development is progressing. From the experience gained during this study, the first callow adult should appear after 198 days' exposure.

It will be noted that 187 days were required before all larvae completed their development at 55 degrees F. No development has been noted at 45° F. and it is believed that this temperature is below the zero of development. Considering the duration of the exposure, the mortality at 50 and 45 degrees F. has been comparatively low.

In order to rectify a few of the mistakes which became evident after the first trials had started, another test was begun. In this, and all succeeding tests, notes were taken on individual larvae, including the dates of the transformation to the prepupa, pupa and callow adult stages for each specimen. As soon as the callow adults formed they were removed from the block to prevent confusion after they had begun to crawl about.

Test No. 3- Started November 23, 1934:

Object: To make a further check on the zero of development and the length of time required to complete pupation at constant temperatures.

Bark: Ponderosa pine from Lassen N.F., collected Oct. 25, 1935, by Bongberg

Container: Bark cells wrapped in cellophane

History: After two weeks in the shade on the Oxford St. lot the larvae were removed and placed in individual cells at lower temperatures.

Summary of Results to date: TABLE NO. 2

-A-		Days after the Start of the Test					
Temperature:	Percent	PUPAE			CALLOW ADULTS		
Degrees F.	Mortality	First	Mean	Last	First	Mean	Last
74	20	9	10	14	13	15	22
65	28	11	18	28	22	27	36
60	20	26	33	48	38	51	60
55	46	51	80	134	77	109	166
50	*	117	*	*	187	*	*

-B- Days after each Larva Reached Prepupal Stage

Temperature Degrees F.	Percent Mortality	PUPAE First	PUPAE Mean	PUPAE Last	CALLOW ADULTS First	CALLOW ADULTS Mean	CALLOW ADULTS Last
74		1	2	4	6	8	11
66		2	3	5	9	12	16
60		2	4	6	16	21	26
55		7	8	11	31	36	44
50		18	*	*	69	*	*

*= Not completed May 8, 1935

Discussion: The first feature of these results, as shown in part -A- is the great number of days intervening between the appearance of the first and last pupa and callow adult after the start of the exposure test--even though the original larvae were carefully selected as to size and apparent maturity.

However, when these transformations are computed from the number of days after each larva reached the prepupal stage, as shown in part -B-, the differences are less striking. Thus, there are two main methods for computing the development through the pupal stage. Figure 1 shows a comparison of these methods for one temperature. It will be noted that in spite of the differences in the number of days between the first and last changes, the curves for these methods closely simulate each other.

Figure 2 shows comparative results of development of Test No. 3 at various temperatures, as figured from the number of days after the prepupal stage was reached.

During the long exposure the mortality at 55° F. and 50° F. has been rather heavy.

With the arrival of samples of bark containing overwintering brood collected in December a new test was started, to check on the clue given in Test 1 and Test 3 -- that the zero of development was between 50° F. and 55° F. (This was three months before the first pupa appeared at 56° F. in either of the previous trials.)

Accordingly, one of the cabinets was adjusted to give a constant temperature of 52.5° F. and larvae placed at this and other temperatures.

Test No. 7 - Started January 19, 1935

Object: To determine whether the zero of development for pupation was closer to 52.5° F. than 50° F.

Bark: Ponderosa pine from Lassen N.F., collected by Bongberg December 19, 1934

Container: Bark cells wrapped in cellophane and black paper

History: Larvae removed January 18, after being on Oxford St. lot since collection, and placed directly into lower temperatures.

Summary of Results todate: TABLE No. 3

-A-	Days after Start of Exposure Test							
Temperature:	Percent	PUPAE			CALLOW ADULTS		
Degrees F.	Mortality	First	Mean	Last	First	Mean	Last
60	10	12	15	18	24	27	33
55	26	28	36	56	52	59	79
52.5	19	18	41	69	45	72	101
50	*	48	*	*	80	*	*

-B-	Days after each Larva reached Prepupal Stage						
60		2	3	6	13	15	19
55		3	6	9	25	30	34
52.5		5	8	11	32	39	52
50		15	*	*	48	*	*

*- Note: Development not completed at 50° F-- May 6, 1935

Discussion: Figure #3 gives a graphic presentation of these results. It will be noted that larvae pupated readily at 52.5° F. and that after a comparatively brief exposure pupae appeared at 50° F. At all of these temperatures, it was observed that once the larvae reached the pupal stage, the first 50% to transform to callow adults did so in a fewer number of days than the last 50%- again pointing to unequal individual development.

It was unfortunate that no sample of this material was placed at 45° F. But, in view of the previous failures to secure pupation below 55° F., it was thought that temperatures lower than 50° F. were below the developmental zero. However, from the above indications nearly anything may happen if given sufficient time.

Early in February, another collection of bark containing overwintering brood was made- this time from the Modoc N.F., but from approximately the same elevation and conditions as the previous samples. Another test was started, using larvae from this material. In addition to the other temperatures, one at 53.6° F. was tried, to determine whether the zero of development was above 52.5° F.

Test No. 10 - Started February 21, 1935

Object: To verify the previous findings in regard to the lessening period of exposure at constant temperatures before pupation began

Bark: Ponderosa pine from Modoc N.F., collected Feb. 6, 1935 by Salman.

Container: Bark cells wrapped in cellophane and black paper

History: Larvae removed Feb. 20, and placed in the 60° F. cabinet overnight, then into lower temperature.

Summary of Results: For some unexplainable reason the larval mortality was considerably higher than in previous tests. Except at 74° F., only a few of the original 50 larvae pupated at any of the other temperatures. The following are the results todate:

TABLE No. 4

-A-		Days after Start of Exposure Test						
Temperature:	Percent :	PUPAE			TALLOW ADULTS			
Degrees F.	:Mortality:	First	Mean	Last	First	Mean	Last	
74	10	1	2	5	9	10	15	
60	80	7	-	-	23	-	-	
55	85	10	-	-	40	-	-	
53.6	50	25	-	-	46	-	-	
50	80	34	-	-	78	-	-	

-B-	Days after each Larva reached Prepupal stage						
74+	-	1	2	3	7	8	10
60	-	3	-	-	19	-	-
55	-	4	-	-	26	-	-
53.6	-	6	-	-	27	-	-
50	-	12	*	*	58	*	*

* — Not complete- May 8, 1935
\- — " enough to give representative results

Discussion: In spite of the heavy mortality in this test, the results clearly indicate that a shorter period of exposure is necessary before pupation begins, depending on the previous conditions endured by the larvae. It was also shown that the zero of development must lie between 45 and 50 degrees, for pupation occurred readily at 50° F.

With the arrival of still another sample of bark containing overwintering brood, the last test was started.

Test No. 14- Started March 28, 1935

Object: To determine the lowest temperature at which larvae subjected to a full winter's conditions would pupate and to check further on the length of exposure before pupation begins.

Bark: Ponderosa Pine from Modoc N.F. collected March 25, 1935 by Salman.

Container: Bark cells wrapped in cellophane and black paper.

History: Larvae removed March 28, and placed directly at lower temperatures-- after careful selection to exclude any that were near the prepupal stage.

Summary of Results todate: While this test is not completed, the results warrant minor conclusions.

TABLE No. 5

-A- Temperature: Degrees F.	Percent Mortality	Days after Start of Exposure Test: PUPAE: First	PUPAE: Mean	PUPAE: Last	CALLOW ADULTS: First	CALLOW ADULTS: Mean	CALLOW ADULTS: Last
74	22	4	6	9	11	12	15
60	*	9	13	18	25	*	*
55	*	10	*	*	34	*	*
52.5	*	10	*	*	38	*	*
50	*	21	*	*	60-?:	*	*

-B-	Days after each Larva reached Prepupal Stage					
74	2	2.5	5	8	8.5	13
60	3	5	7	19	*	*
55	5	*	*	29	*	*
52.5	6	*	*	34	*	*
50	12	*	*	50-?:	*	*

* — Not complete- May 6, 1935

Discussion: While no development has yet been observed at either 45° F. or 41° F., only 21 days' exposure were required before pupation began at 50° F., as compared with 166 days for pupation to start in the first test. This seems to indicate that some development may be expected at lower temperature from this later material. The first callow adult has not yet appeared at 50° F. The numbers shown above have been computed and based on previous observations.

B. Effect of time and temperature upon larvae from bark collected at different intervals during the winter and subjected to the same constant temperatures.

As each test succeeded the other, it became more and more evident that larvae in the overwintering condition, when subjected to constant temperature, had varying requirements for pupation depending on the length of exposure to normal winter conditions.

Table No. 6 presents a summary of the comparative effects of time and temperature on larvae collected at different periods during their overwintering condition.

TABLE No. 6

Number of Days after each Larva reached Prepupal Stage

Temperature	Oct. Bark First	Oct. Bark Last	Oct. Bark-a First	Oct. Bark-a Last	Dec. Bark First	Dec. Bark Last	Feb. Bark First	Feb. Bark Last	Mar. Bark First	Mar. Bark Last
PUPAE										
74+	7	16	9	14	No sample		4	9	4	9
60	23	55	26	48	12	18	8	16	8	*
55	14		51	134	28	59	19	32	8	*
50	156	*	117	*	48	*	34	*	21	*
CALLOW ADULTS										
74	14	22	13	22	No sample		10	16	11	15
60	40	84	37	60	24	33	23	31	24	*
55	42		77	166	52	82	40	58	35	*
50	196	*	167	*	80	*	78	*	60	*

a- Remained out doors 2 weeks longer than first sample
*- Not completed May 6, 1935

A more striking picture of these results is obtained from Figures 4 and 5, which emphasizes the appearance of the first pupa and callow adult--especially that of the slower development at 50° F.

These results tend to show

(1) That these insects follow the calendar rather closely. The 156 days and 117 days required before the first pupa appeared at 50° F. from the October bark, and the 48 and 34 days for the winter broods all fall on about the same calendar days, March 21-25.

(2) That a certain amount of feeding and development takes place during the winter which appears to be necessary for normal pupation.

(3) That overwintering larvae are ready to pupate and require a shorter period to complete this transformation in the spring than those larvae in the early fall or mid-winter condition.

Relation of Temperature to Advanced Brood Stages

A - Mature Larvae:

It has often been noted that pupal forms are rarely, if ever, found during the winter, but that mature larvae enter the winter as larvae in a more or less dormant condition or as prepupal larvae. This pointed to the fact that there was a temperature that controlled pupation which differed from that required for larval and pupal development.

That it is the prepupal stage in the fall of the year which is the bar to pupation at temperatures near the zero of development has been quite clearly demonstrated during these experiments.

Overwintering mature larvae from the fall brood after 175 days at 45° have shown no signs of pupation. After 156 days at 50° F. the first pupa has appeared. Often during this long period these larvae appeared to be in a prepupal stage and nearly ready to pupate, but no further development took place. This behavior led to the belief that it was the change from the mature larva to the prepupal condition which prevented pupation at those temperatures near the zero of development. Additional evidence to support this belief was shortly forthcoming from two rather simple tests. One will be discussed under Prepupal Larvae and the other under Pupae.

B - Prepupal Larvae:

The first of these two tests was as follows:

Test No. 8 - Started January 22, 1935

Object: To determine whether the prepupal stage was the bar to pupation at the lower developmental temperatures.

Bark: Ponderosa pine from Lassen N.F., collected December 10, 1934, by Bongberg.

Container: Bark cells wrapped in cellophane and black paper.

History: Mature larvae removed from the bark and placed directly into the 74° cabinet. Just as soon as the first signs of the prepupal condition appeared they were placed in bark cells and taken to the following temperatures: 60° F., 55° F., 53.6° F., 52.5° F., 50° F., 45° F., 45° F., 41° F., 36° F., 32° F., and 20° F.

Summary of Results todate:

TABLE No. 7

Temperature Degrees F.	Original Number	Percent Mortality	Number of Days after each Larva reached Prepupal Stage: PUPAE First	PUPAE Mean	PUPAE Last	CALLOW ADULTS First	CALLOW ADULTS Mean	CALLOW ADULTS Last
60	100	8	4	5	7	15	17	20
55	168	7	4	6	12	27	31	35
53.6	193	9	4	7	11	26	30	35
52.5	98	16	4	7	13	33	38	44
50	122	11	5	8	15	38	46	62
45	97	*	12	21	42	79	*	*

* - Not completed May 6, 1935

Discussion: While it was entirely logical and expected that larvae thus stimulated would complete their pupation at temperatures above 50° F., the reaction of larvae at 50° F. and below were the most interesting. For up to this date no development had been noted at 50° F. and it appeared that it was the physiological change from the mature larval to the prepupal condition which prohibited pupation. And here were larvae raised to this prepupal stage readily transforming to pupae at 50° F. and in short order at 45°F. All of which supported the original idea of this prepupal bar. Figures 6 and 7 show these results.

Once this bar has been broken, the lower temperatures at which pupation will take place are still unknown. There have been 7 pupae appear at 41° and 1 at 36° F. Four of those at 41° and the 1 at 36° came after a relatively few days of exposure indicating that the prepupal larvae were slightly more advanced than originally supposed. However, further development is anticipated after a longer period at 41°. Below this temperature, the chances for development to take place appear doubtful, as mortality is increasing with the length of exposure.

C - Pupae:

The second test which demonstrated the importance of the prepupal stage was one in which pupae were used.

Test No. 9- Started January 27, 1935

Object: To determine the lowest temperature at which pupae would transform to Callow Adults.

Bark: Ponderosa pine from Lassen N.F., collected December 10, 1934, by Bongberg.

History: Mature larvae removed from the bark and placed in the 74° cabinet where they were carried over the prepupal condition. Just as soon as the pupal stage was reached they were placed in bark cells and taken to lower temperatures of 60°, 52.5°, 50°, 45°, 41°, 36° and 20° F.

Summary of Results todate: TABLE NO. 8

Temperature Degrees F.	Number of Pupae	Percent Mortality	Number of days after each Larva reached Pupal Stage: CALLOW ADULTS First	Mean	Last
60	74	3	10	15	17
52.5	50	2	22	28	36
50	78	5	28	36	46
45	92	*	78	*	*

* Not completed May 6, 1935

Discussion: Since this test, as well as No. 2, was started before development began at 50° F. in the other tests, the results were a little surprising. Callow Adults appeared readily at 50° F. after only 28 days' exposure. After 78 days the first successful transformation was completed at 45° F. And, at the present time, after 80 days' exposure at 41° F. 8 pupae show slight coloration, indicating development. Below this no development has been noted. These results are graphically shown in Figure 8.

The transformation from pupa to adult, especially at lower temperatures is not always successful. This fact was noted during nearly all of the tests and also has been noted by Beal (1). It was very evident during this test at 45° F.

The first pupa broke the pupal skin at 45° F. after 52 days' exposure--but it was not perfectly formed. The body attained the normal callow adult color, the mouth parts were moveable, the legs unfolded from their pupal position and were normal, the elytra, however, failed to develop perfectly. Instead of covering the second pair of wings on the dorsal surface, they remained fastened to the ventral surface, and in this condition the insect usually died after breaking the pupal skin. The first normal callow adult appeared after 78 days' exposure.

While the percentage of successful transformations from Pupae to Callow Adults at 45° has been relatively small todate, the fact that some larvae will pass through their pupal stage and emerge as callow adults is rather striking.

All of which upholds the statement that it appears to be the prepupal stage which bars pupation at lower temperatures.

D - Normal and Undernourished Larvae:

During the process of securing large 4th instar larvae for these tests, many similar individuals were removed from the same positions of the outer bark. Measurements of the head capsules showed these to be of the same instar--the 4th--but evidently undernourished.

Two tests were started using these undernourished larvae which will be summarized together.

Test 1 and 5- Started November 10, and 23, 1934.

Object: To determine the behavior of undernourished larvae at constant temperatures.

Bark: Ponderosa pine from Lassen N.F., collected October 25, 1934, by Bongberg.

History: Larvae removed from the bark and placed at lower temperatures.

Summary of Results todate: TABLE NO. 9

Temperature: Degrees F.	Percent Mortality	Days after Start of Exposure: PUPAE First	PUPAE Last	CALLOW ADULTS First	CALLOW ADULTS Last
74	62	9	19	14	24
65	78	15	30	27	43
60	45	22	41	36	59
55	82	50	131	78	156
50	*	119	*	*	*

* -- Not completed May 6, 1935

Discussion: In spite of a high mortality small undernourished larvae follow the same pupation tendencies as normal larvae. A comparison of the relative sizes of these larvae, pupae and adults with normal individuals is shown in Plate No. 6. Since only the large, mature larvae were used from the other samples of bark, no comparative results are available.

Effect of Relative Humidity on Pupation

While it seems logical that temperature exerts the dominant influence on larval activity, two tests were made to determine the effect of high and low relative humidities on pupation. In the first test many larvae burrowed beneath the surface of the bark cells and were lost from view, giving too few figures to be of value. The second attempt gave better results.

Test No. 12 - Started March 30, 1935

Object: To determine the effect of various humidities on pupation.

Bark: Ponderosa pine from the Modoc N.F., collected March 25, 1935, by Salman.

History: Larvae removed from the bark and placed in bark cells wrapped in cellophane and black paper. These cells were then placed on a small rack in a closed battery jar at varying relative humidities and all jars placed at 74° F. (Plate No. 7.)

Summary of Results: TABLE NO. 10

Percent of: Humidity	Agent	Percent Mortality	Number of Days after Start of Test: PUPAE First	PUPAE Last	CALLOW ADULTS First	CALLOW ADULTS Last
100	H_2O	4	4	8	10	15
75	NaCL	9	3	9	10	13
65	$Ca(NO_3)_2\ 4H_2O$	10	4	9	8	13
20	CH_3 COOK	40	4	10	9	19

Discussion: Carried out, as it was, at 74° F.— at which temperature development is very rapid-- this test probably does not represent the effects of these humidities. It is conceivable that a similar test made at a lower temperature would more nearly show the effects of humidities.

The most noticeable features are the high mortality and longer period of development at 20% humidity. Over half of the mortality occurred in the pupal stage; 21% of the adults had poorly developed wings--the elytra remained fastened to the ventral surface of the body and, although the legs moved freely and the body started to color normally, they soon died.

Much of the difficulty with the green mold which enevitably accompanies high humidities was overcome by subjecting the bark cells, used for the 100% test, to steam at 15 pounds pressure for 30 minutes in an autoclave. This checked the development of mold quite satisfactorily.

MOUNTAIN PINE BEETLE STUDIES

Dendroctonus monticolae, Hopk.

The experiments to determine the temperature requirements for pupation of the Mountain pine beetle have not been as successful as those with the previously discussed species. Being notorious for their lack of uniformity and consistency of development, they have behaved true to form during this project.

Review of Previous Work:

Very little work has been done with temperatures near the developmental zero of this insect. Smith (2) exposed larvae and pupae to constant temperatures of 40, 36, and 0 degrees F. This material was not sufficient to warrant conclusions but gave a few indications.

At 0° F. the mortality was 100%. At 40° and 36° F. mature larvae showed no development. A few prepupal larvae became pupae at 40° F. Pupae in the more advanced stages transformed to adults at 40 and 36° F.

Equipment and Methods: The larval material for this work was obtained from Sugar pine logs upon which a forced attack had been made in the field. The logs were brought to Berkeley and placed out of doors until the larvae reached maturity.

The larvae were placed in the same temperature compartments with the Western pine beetle. (Plate No. 2.)

Containers:

In the previous work, the larvae were placed in large cells made in plaster of paris blocks. Each cell holding 10 to 20 larvae. Another test

was made in which the larvae were left undisturbed in their natural habitat of cambium layer of Sugar pine logs and the development noted after a long period of exposure. While neither of these methods gave very satisfactory results, some improvements were made.

In order to isolate the larvae and to follow individual development, smaller plaster of paris blocks were made. Fifty shallow holes were drilled with a 1/4 inch drill and covered by thin glass plates. (Plate No. 8.) These blocks were placed in a closed tin can over moist cotton in order to maintain the high moisture content necessary for their development. However, nearly all of the larvae were killed by the mold and fungi which inevitably accompanies work with high humidity.

An attempt was made to keep the larvae in their natural surroundings and yet follow their development. Small sections of infested Sugar pine logs were cut. The exposed wood was given a thick coat of paraffin to preserve the moisture. Then the bark was carefully lifted from the section. All adults, pupae and prepupal larvae were removed--leaving only mature and a few immature larvae. The bark was carefully replaced and held tightly in place by strong rubber bands. (Plate No. 9) By placing three or four of these sections in a temperature compartment, the development of 40 or 50 mature larvae could be followed.

These sections have worked quite satisfactorily at the lower temperatures. After four months' exposure at temperatures below 50° F., they have remained quite moist, with only a minimum of fungi. The mortality has been very light. At temperatures above 55° F. the cambium tended to dry out after long exposures and the bark pulled slightly away from the wood.

Relation of Temperature and Brood Stage to Pupation

A. Plaster of Paris Blocks:

(1) Mature Larvae-- Because of the Heavy mortality caused by mold and fungi, only a few of the original larvae transformed to adults, and no percentages have been figured. The following table gives the average results of those mature larvae that reached the adult stage.

TABLE NO. 11

Temperature: Degrees F.	Number of days after reaching prepupal Stage until the average Transformation occurred:- PUPAE	GALLOW ADULTS
74	3	8
60	4	20
55	5	36
50	8	50

Below 50°F. no development of mature larvae was observed.

(2) Prepupal Larvae-- Larvae in the prepupal condition transformed to pupae and to callow adults at temperatures from 74 to 50° F. At 45 and 41° F., larvae in this condition reached the pupal stage but were killed by fungi. At 36 and 32° F. no development occurred.

(3) Pupae-- Callow adults appeared from early pupae (all white) at temperatures as low as 41° F. At 36° F. the pupae were unable to break the pupal skin and did not become perfect adults. At 32° F. none developed.

Pupae that were more advanced (showing body color) reached the Callow adult stage at temperatures as low as 36° F. One transformed at 32° F. but was probably more advanced than the others.

B. Sugar pine sections:

Only mature larvae were left in these log sections when they were prepared for exposure to lower constant temperatures.

The development of these larvae from 74° to 50° F. followed closely that of the larvae in the plaster of paris cells. At 50° F., however, the development progressed at a much slower rate and only a few reached the Callow adult state. Below 50° F. practically no development has taken place after 120 days of continuous exposure. There were 2 pupae appearing at 45° F. after 32 days' exposure-- probably from larvae near the prepupal stage at the time they were placed. One of these pupae died and the other reached the callow a adult stage. Except for this, no development has taken place.

Discussion: From the meager results obtained from the above data, it appears that this species follows some of the tendencies that were observed with the Western pine beetle, more closely than had been realized before.

(1) For practical purposes the zero of development for larvae appears to be close to 50° F.

(2) It appears that the change from the mature larval to the prepupal condition acts as a bar to pupation at lower temperatures.

(3) That once this bar is broken the transformation to the pupa and adult stages will continue at temperatures as low as 36° F.

SUMMARY AND CONCLUSIONS

From September, 1934 to May, 1935, an attempt has been made in the laboratory to determine the temperature requirements for pupation of the Western pine beetle and Mountain pine beetle. This study has been carried out on a part-time basis directly supervised by Mr. J. M. Miller and Dr. K. A. Salman of the U. S. Bureau of Entomology and Plant Quarantine. The project was made possible by a special fund of the College of Agriculture of the University of California.

The Western Pine Beetle:

During this pupation study attention has been centered on the elimination of as many variable factors as possible. A brief summary of these attempts is as follows:

1. Larvae from one locality- With two exceptions larvae were removed from samples of Ponderosa pine bark collected from the Lassen National Forest. The two exceptions being bark from the Modoc National Forest. However, conditions on these two areas are similar.

2. Selection of larvae- All larvae were in an overwintering condition and after removal from the bark were sorted for size. Only normal mature, 4th instar larvae were selected.

3. Containers- When sorted the larvae were placed in individual cells made in plates of outer bark which simulated their natural habitat very closely.

4. Protection- These bark cells were wrapped in cellophane and black paper to exclude direct contact with the environment.

5. Temperature- Constant temperatures of 74, 65, 60, 55, and 50° F., which varied less than half a degree, were maintained in small portable cabinets. For lower temperatures, space in larger rooms was available. Here the temperatures varied 2 or 3 degrees for 20 or 30 minutes during defrosting operations.

6. Humidity- The relative humidity was stabilized at 55-65%. This correlated with the 12% moisture content as found in samples of outer bark.

7. Data- Notes were taken as near the same time each day as possible, on the transformations and development of individual larvae.

Conclusions:

1. There appears to be no temperature which may be called the zero of development that will cover all stages of the western pine beetle at different seasons of the year.

2. The zero of development depends upon:

   (a) The brood stage--whether larva, prepupa, or pupa.

   (b) The time of the winter during which larvae are removed from their natural overwintering habitat.

3.For mature larvae removed during the early fall--October--the zero of development lies above 50° F. and probably closer to 55° F. However, after 156 days' exposure, development began at 50° F.

4.For mature larvae removed during the early spring (February and March), the zero of development appears to lie below 50° F., probably closer to 45° F.

5.Prepupal larvae will transform to pupae and to callow adults at 45° F. After the prepupal stage has been reached, an average of 20-25 days is required for the transformation to the pupal stage. After 95 days' exposure callow adults begin to appear at this temperature.

6.Pupae transform to callow adults after 85 days' exposure at 45° F. and indications are that this change will take place at 41° F. after a longer period of exposure.

7.It appears that the physiological change from mature larvae to prepupal larvae prohibits pupation at lower temperatures. Once this bar has been broken, metamorphosis will continue slowly at relatively low temperatures.

8.A certain amount of feeding and development appears to be necessary during the winter which enables the larvae to transform rapidly to pupae and thence to callow adults with the rise in temperatures in the spring.

9.At low humidities, pupae have difficulty in transforming to perfect adults. The elytra remain attached to the ventral surface and fail to cover the second pair of wings.

The Mountain Pine Beetle:

The results with this species have not been successful.

1.Larvae- All larvae were collected from Sugar pine logs, upon which a forced attack had been made, and were selected as to size and maturity.

2.Containers- Small plaster of paris blocks with individual cells were tried. Larvae were also left in their natural habitat in small sections of Sugar pine which were paraffined to conserve the moisture. The bark was removable and was held in place with strong rubber bands.

3.Temperature- The same temperature compartments that were employed for the Western pine beetle were used for this insect.

4.Humidity- Was maintained at 100% but heavy mortality resulted from mold and fungi. At humidities of 50-60%, the larvae appeared to die of desiccation.

Conclusions:

1. At constant temperatures below 50° F., mature larvae failed to transform to pupae.

2. It appears that the change which prohibits pupation of the Western pine beetle at lower temperatures also affects this species--that is the physiological change from mature to prepupal larvae.

3. Once this bar has been broken, transformation to pupal and callow adult stages occur at temperatures as low as 36° F.

4. The reactions of mature larvae in artificial containers are not the same as those under more natural conditions.

Recommendations:

If in the future additional experiments are conducted with these insects, a few pertinent suggestions may not be out of place.

1. Due to the apparent complexity of this phase of their life history, only one species should be experimented with at one time.

2. Experiment with temperatures below 55° F. in 2.5 degree intervals, since development above this point progresses quite rapidly and below this point very slowly.

3. Samples of bark containing overwintering brood should be collected at intervals during the winter.

4. Samples of insects from this bark should be subjected to all temperatures available, in

   (1) the active mature larval stage

   (2) At the beginning of the prepupal stage

   (3) At the beginning of the pupal stage

   in order to arrive at the zero of development for each stage.

5. A test should be made to determine the effect of relative humidities on pupation. This should be made at a temperature of about 60° F., since development is slower and the full effect of humidity could be observed.

6. Experiments with the Mountain pine beetle should not be made at humidities of 100%, due to the loss from mold and fungi.

While definite and rigid conclusions cannot be drawn from the above data, it is hoped that this endeavor will be of some aid in future attempts to determine the temperature requirements for pupation of these two species.

Respectfully submitted,

JOHN M. WHITESIDE

May 20, 1935

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BIBLIOGRAPHY

(1) Beal, J. A. 1931 — Relation of Temperature and Moisture to Brood Development of the Western pine beetle. Manuscript report on file with U.S.Bureau of Entomology and Plant Quarantine

(2) Smith, G.L. 1934 — Unsummarized results of laboratory studies. Notes of the U.S.Bureau of Entomology & Plant Quarantine

(3) Hatfield, Irg 1931 — Control of Moisture Content of Air and Wood in Fresh-Air Chambers. Jour. Agri. Res.- Vol. 42, No. 5; p.p. 301-5

(4) Spencer, H.M. 1926 — Laboratory Methods for Maintaining Constant humidities. International Critical Tables Vol. 1, p. 67

(5) Sweetman, M.L. 1933 — Studies of chemical control of Relative Humidity in closed spaces Ecology, Vol. 14, No. 1, p. 40

(6) Buxton, P.A. and Mellanby, K., 1934 — The Measurement and control of Humidity. Bul. Ent. Res. Vol. 25, part 2, p. 171

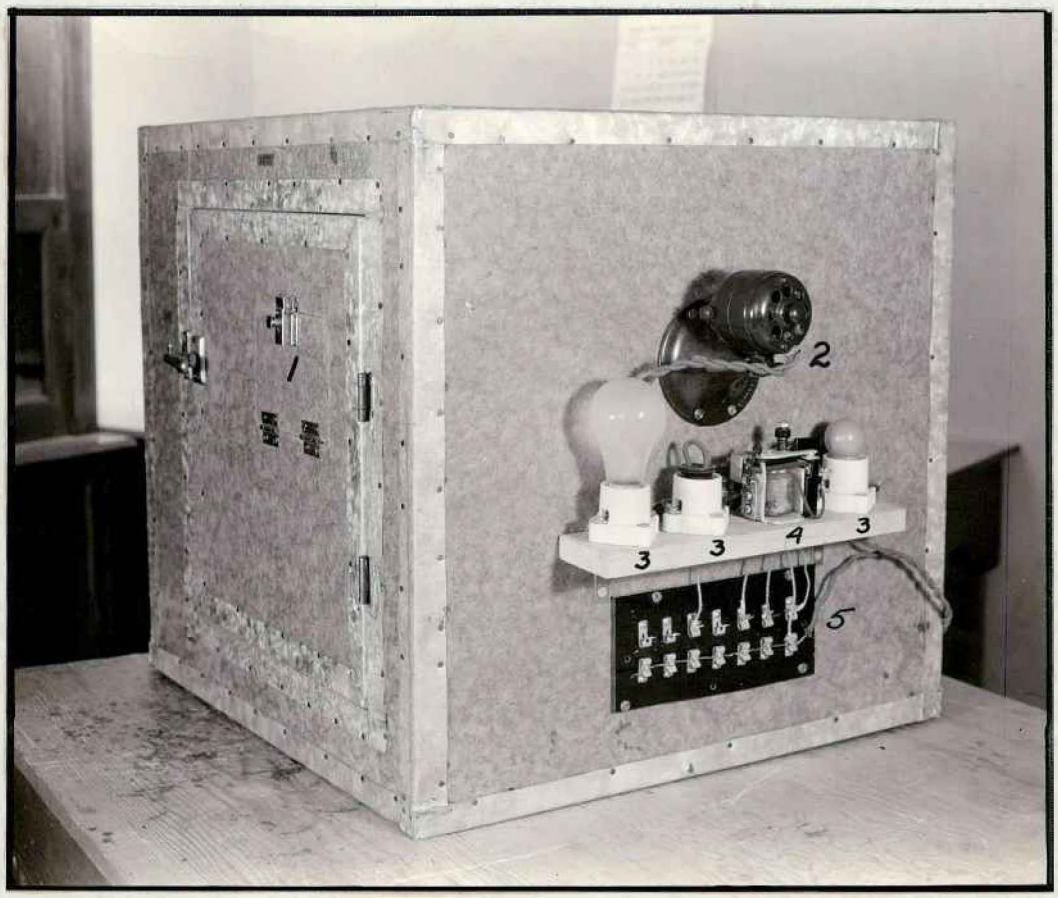


Plate No. 1

Portable cabinet equipped for constant temperature control. Made of Masonite with outer dimensions of 22" x 22" x 32" and inner dimensions of 18" x 18" x 18"

(1) Small observation door
(2) Motor and mounting for 6 inch fan
(3) Lamps used for resistance with the fan and thermo-regulator
(4) Relay switch
(5) Wiring panel

Photo No. 9291 - B

By Patterson

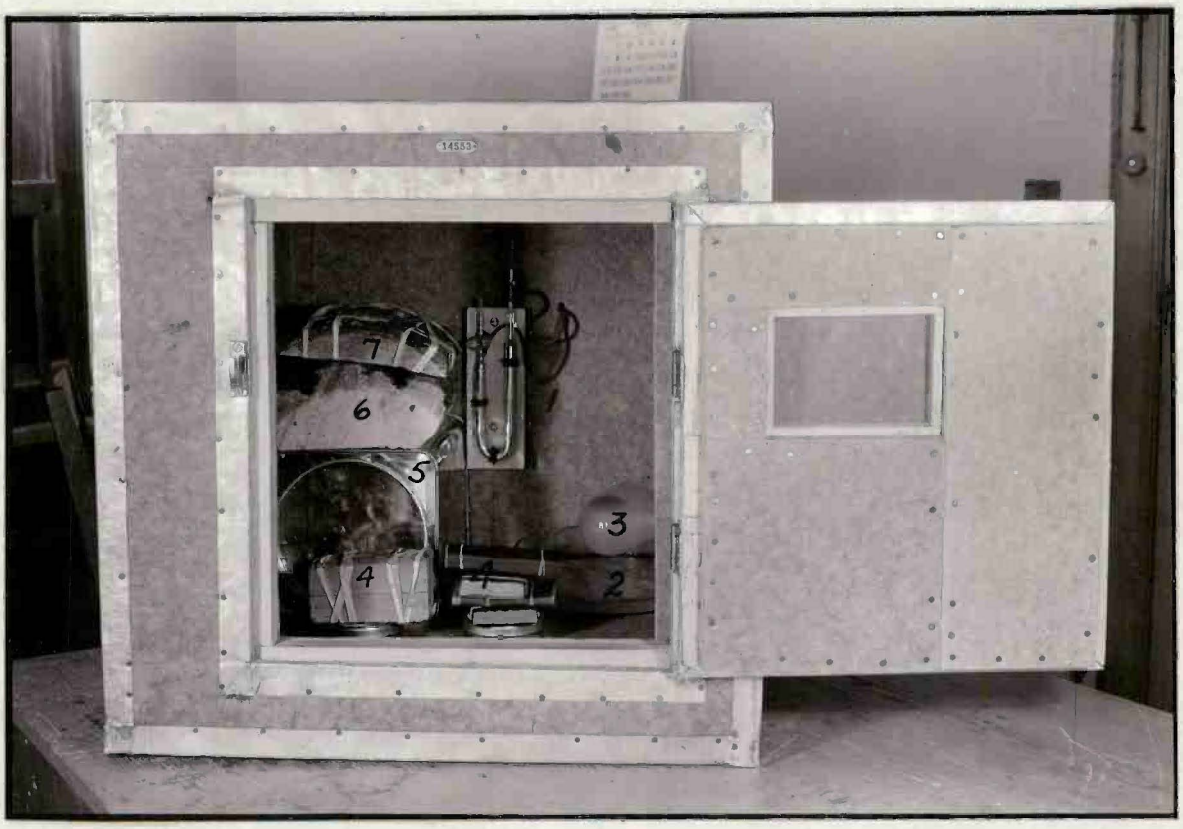


Plate No. 2

Interior view of constant temperature cabinet.

(1) Mercury "U" tube thermo-regulator
(2) Open dish of super-saturated Calcium nitrate solution to maintain moisture content of 55% - 65%.
(3) Lamps used to raise and maintain constant temperature.
(4) Bark cells containing D. brevicomis Lec.
(5) Closed tin with plaster of paris block holding mature D. monticolae Hopk. with humidity at 100%.
(6) Ponderosa Pine block containing eggs and small larvae of D. brevicomis Lec.
(7) Sugar Pine block containing mature D. monticolae Hopk.

Photo No. 9291-A By Patterson

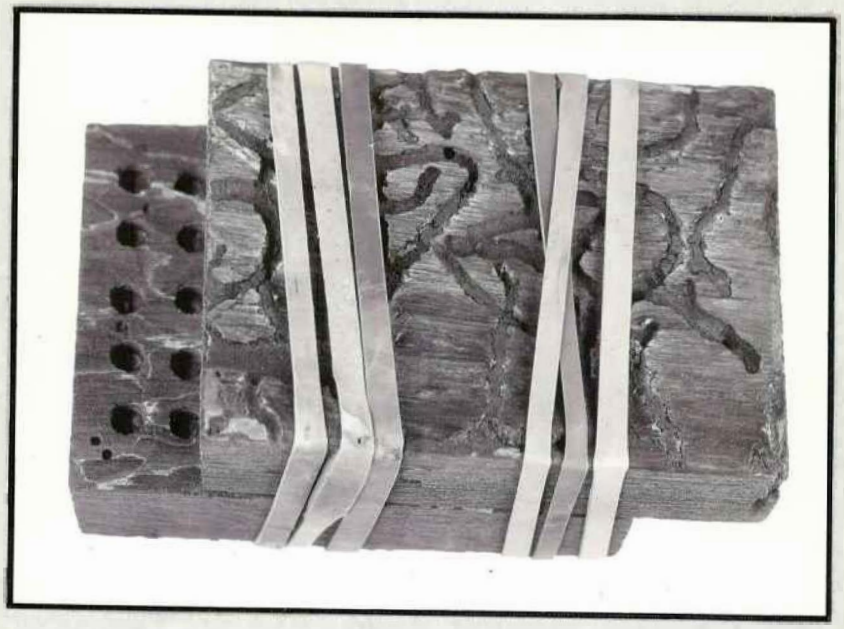


Plate No. 3

Thick section of Ponderosa Pine bark with individual cells and thick bark cover held in place with strong rubber bands, used in D. brevicomis pupation study.
Photo No. 9293 - C By Patterson

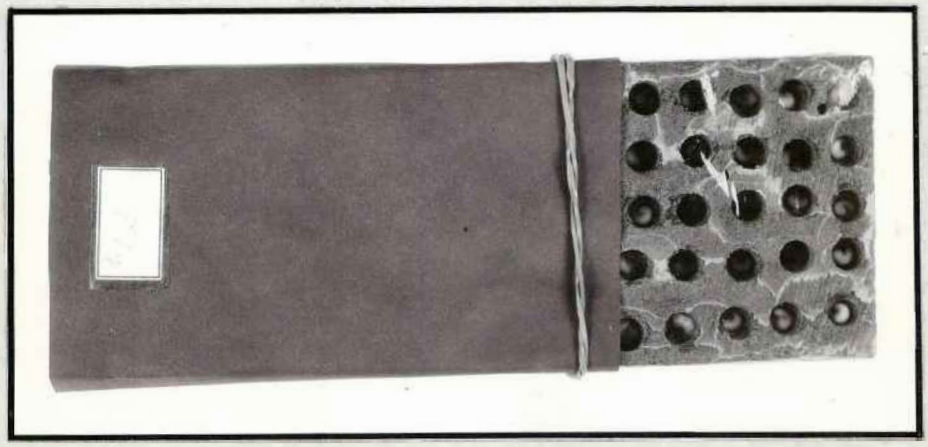


Plate No. 4

Mature D. brevicomis Leo larvae in individual cells in thinner sections of Ponderosa Pine bark wrapped in cellophane and black paper held in place with rubber bands. This type container was used in nearly all of the pupation tests.
Photo No. 9293-E By Patterson

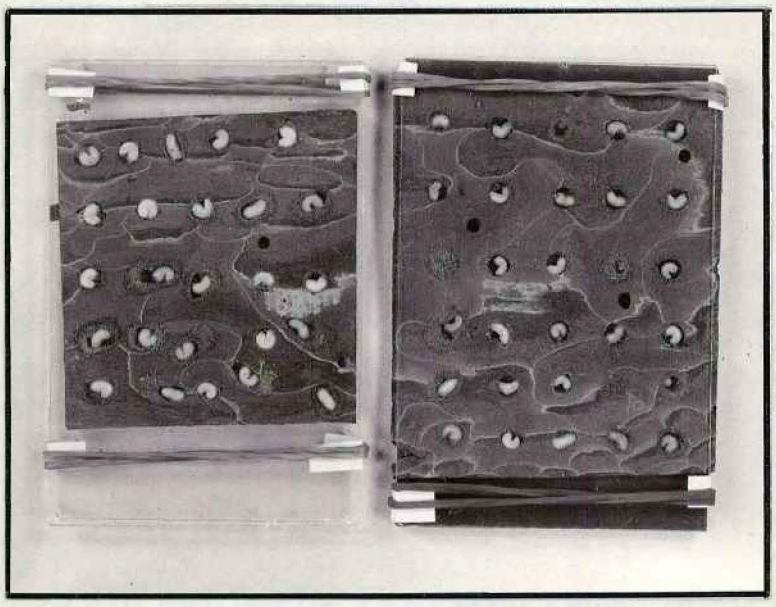


Plate No. 5

Mature D. brevicomis Lec. in bark 1 to 1½ millimeters thick between glass plates. Note that a few larvae have worked beneath the surface and that many have enlarged the original oval cell to an oblong cell prior to pupation.

Photo No.,9293-B By Patterson

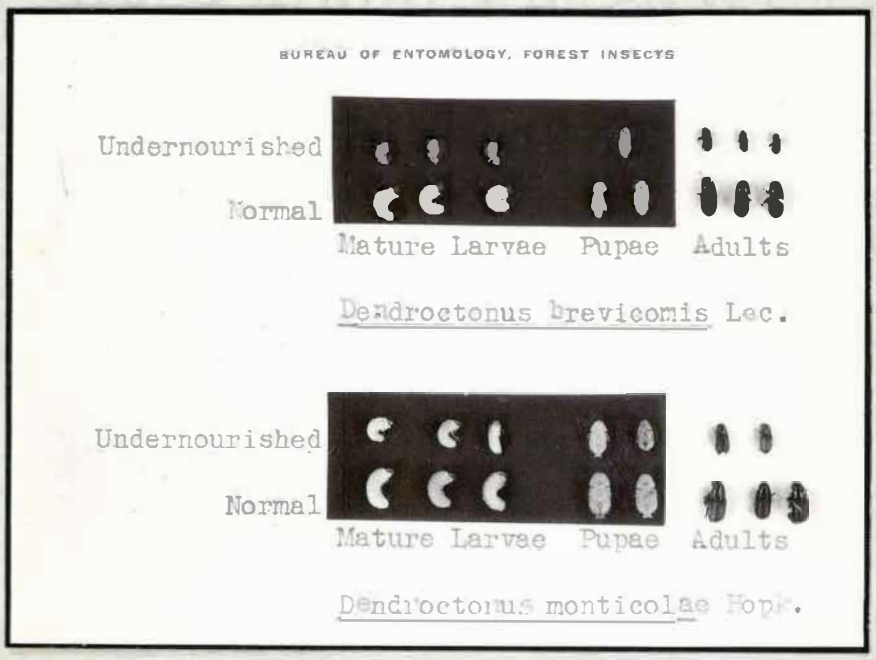


Plate No. 6

Although removed from the same portions of the bark, some larvae are of normal size and others are apparentlym under-nourished. This shows the differences in the sizes of the pupae and adults developing from these mature larvae. All are shown natural size.

Photo No. 9294 **By Patterson**



Photo No. 7

Showing a closed battery jar with bark cells containing mature D. brevicomis Lec. larvae suspended over a super-saturated Calcium nitrate solution used in the humidity test.

Photo No. 9292 By Patterson

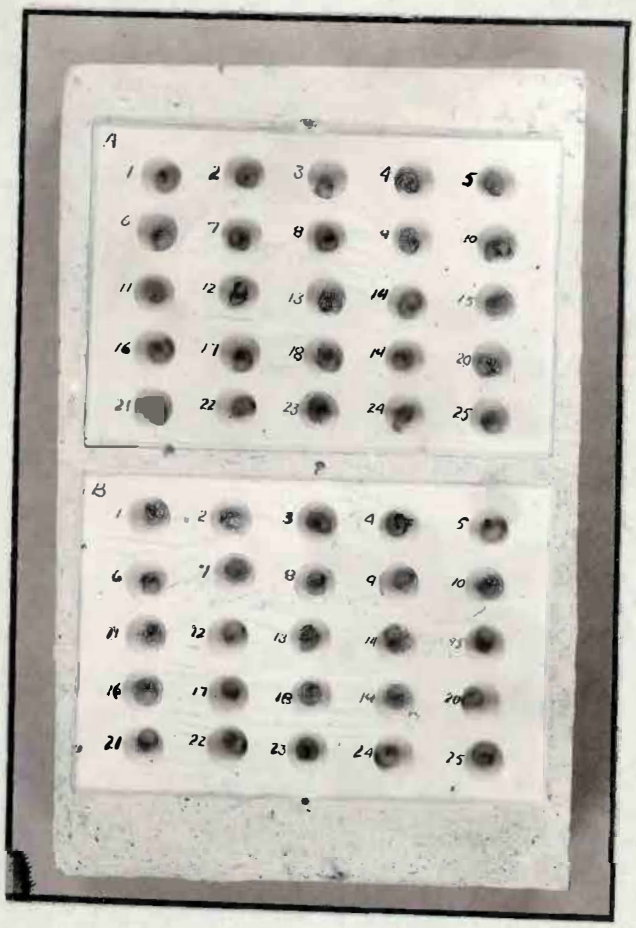


Plate No. 8

Plaster of paris block with individual cells covered by glass plates, used for D. monticolae Hopk. pupation study.
Photo No 9295-D By Patterson

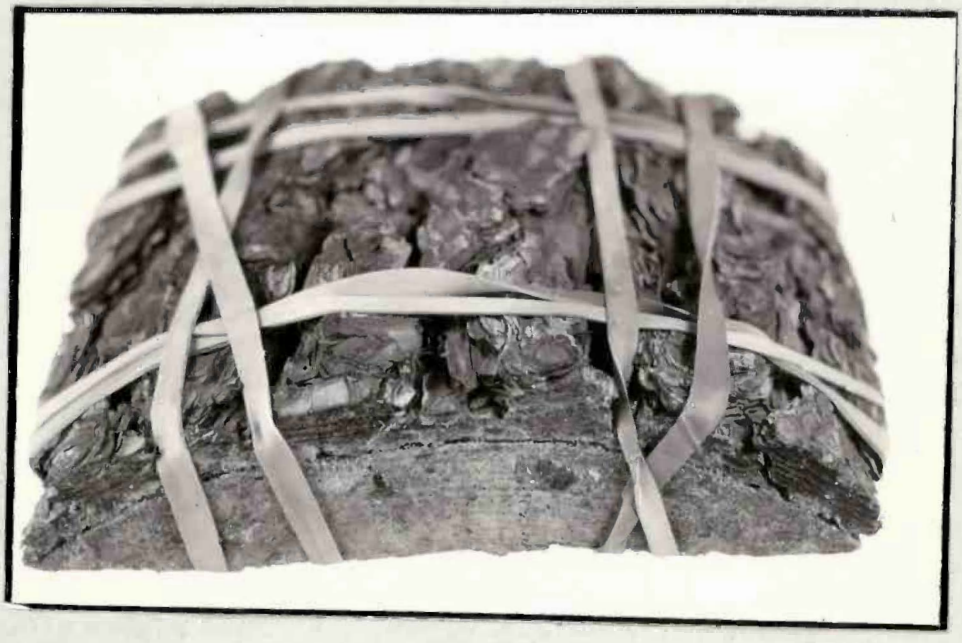


Plate No. 9

Section of Sugar Pine log containing mature D. monticolae Hopk. used in pupation study. Observation is made by lifting the bark from the wood. The strong rubber bands hold the bark tightly against the wood.
Photo No. 9293-A By Patterson



Plate No. 10

Showing the color changes which occur at 74 degrees F. during the pupal stage of *D. brevicomis* Lec.

(1) Newly formed pupa - void of color
(2) Pupa 1 day old - eyes light yellow
(3) Pupa 2 days old - eyes light brown
(4) Pupa 3 days old - eyes dark brown
(5) Pupa 4 days old - eyes black, mandibles dark brown
(6) Pupa 5 days old - eyes black, mandibles black, tips of second pair of wings black
(7) Pupa 6 days old - eyes black, mouth parts black, vertex white, pronotum golden yellow, second pair of wings black, ventral sclerites light yellow
(8) Newly formed Callow Adult - eyes black, mouth parts black, vertex white, pronotum golden yellow, elytra white, second pair of wings black, legs yellow, ventral sclerites yellow

Four times natural size

Photo by Patterson

FIG. NO. 1

A COMPARISON OF THREE METHODS FOR SHOWING THE APPEARANCE OF THE FIRST AND LAST PUPA AND CALLOW ADULT OF D. MELANOGASTER LEC. FROM OCTOBER BANK AT 60°F.

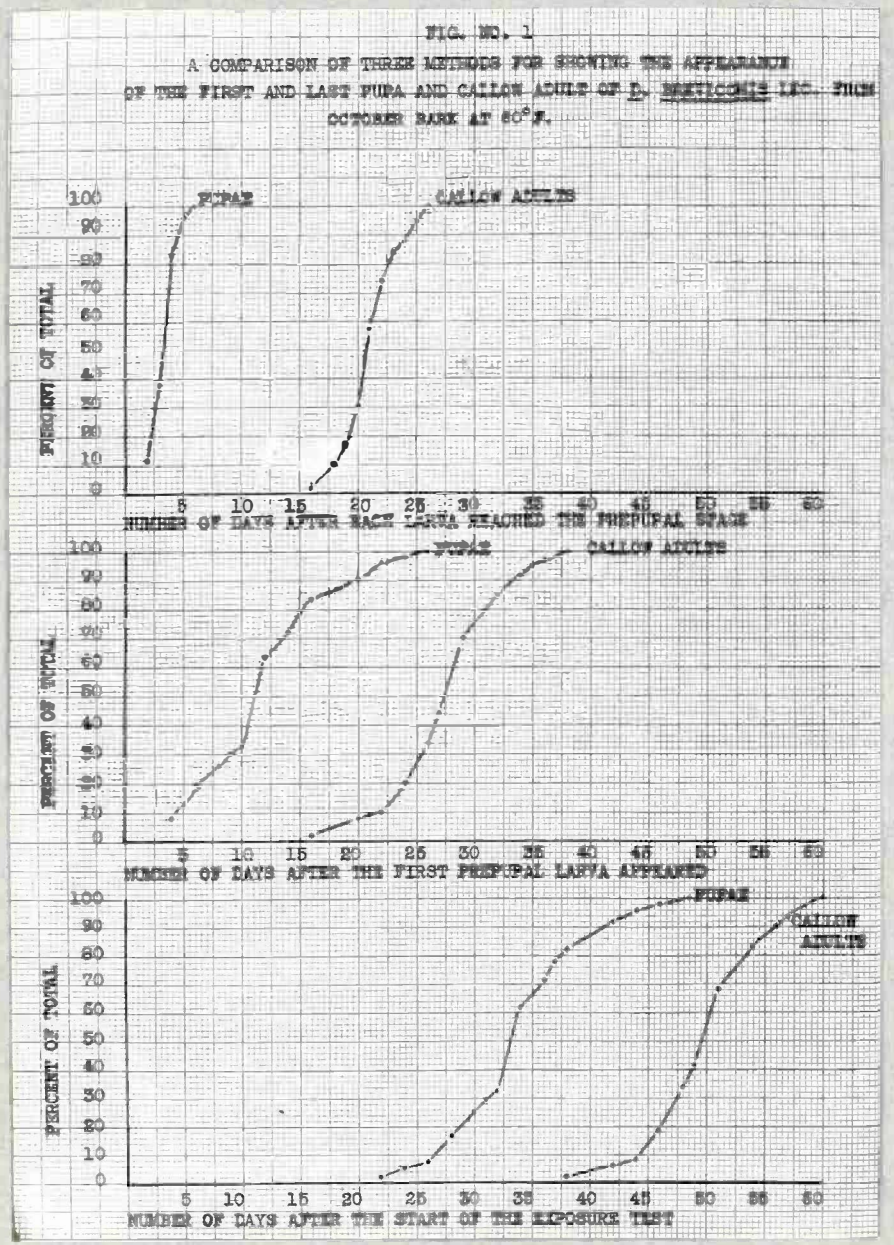


FIG. NO. 2

TEST NO. 2. THE EFFECT OF TEMPERATURE ON THE PUPATION OF D. MELANOGASTER LEC. FROM OCTOBER BANK, SHOWING THE APPEARANCE OF THE FIRST AND LAST PUPA AND CALLOW ADULT AFTER THE BEGINNING OF THE PREPUPAL STAGE.

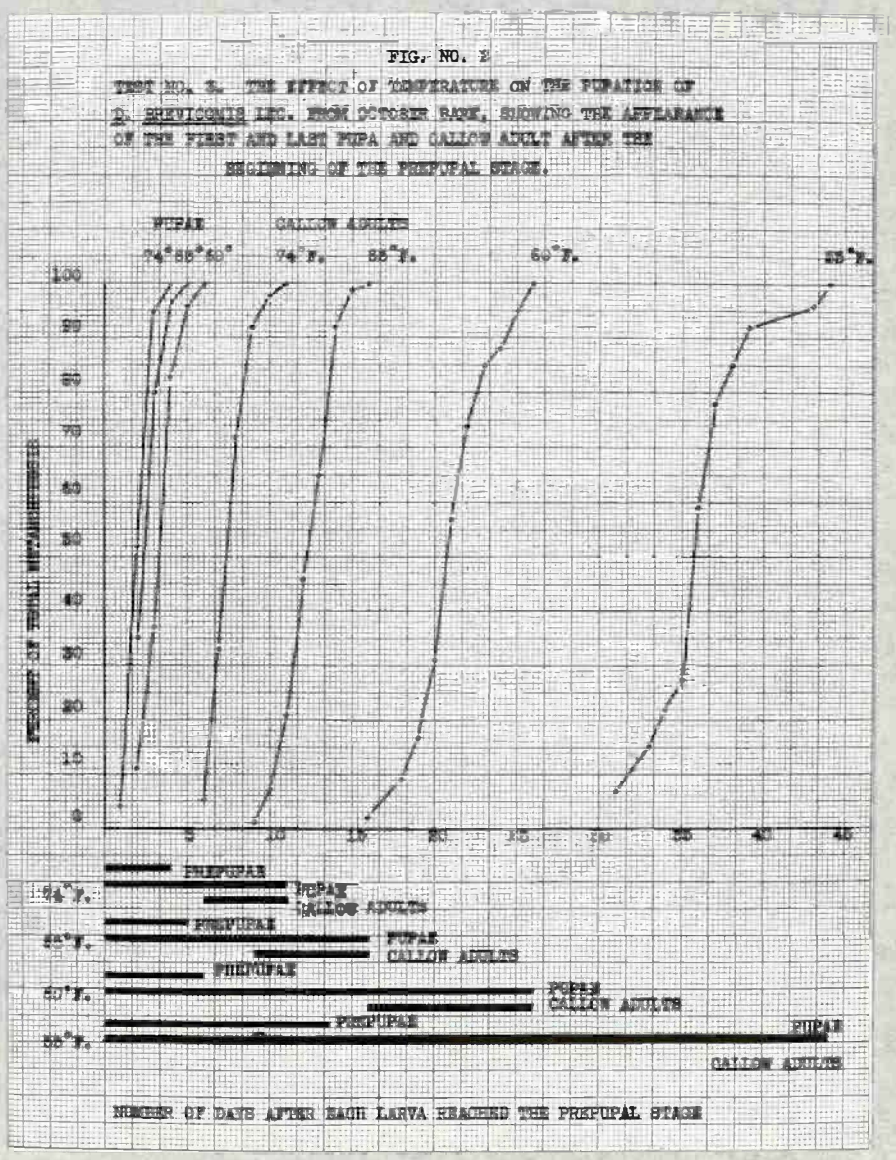


FIG. NO. 5

TEST NO. 7. EFFECT OF TEMPERATURE ON THE PUPATION OF D. SEPTICOSUS LEC., FROM DECIDUOUS BARK.

SHOWING THE APPEARANCE OF THE FIRST AND LAST INDIVIDUALS

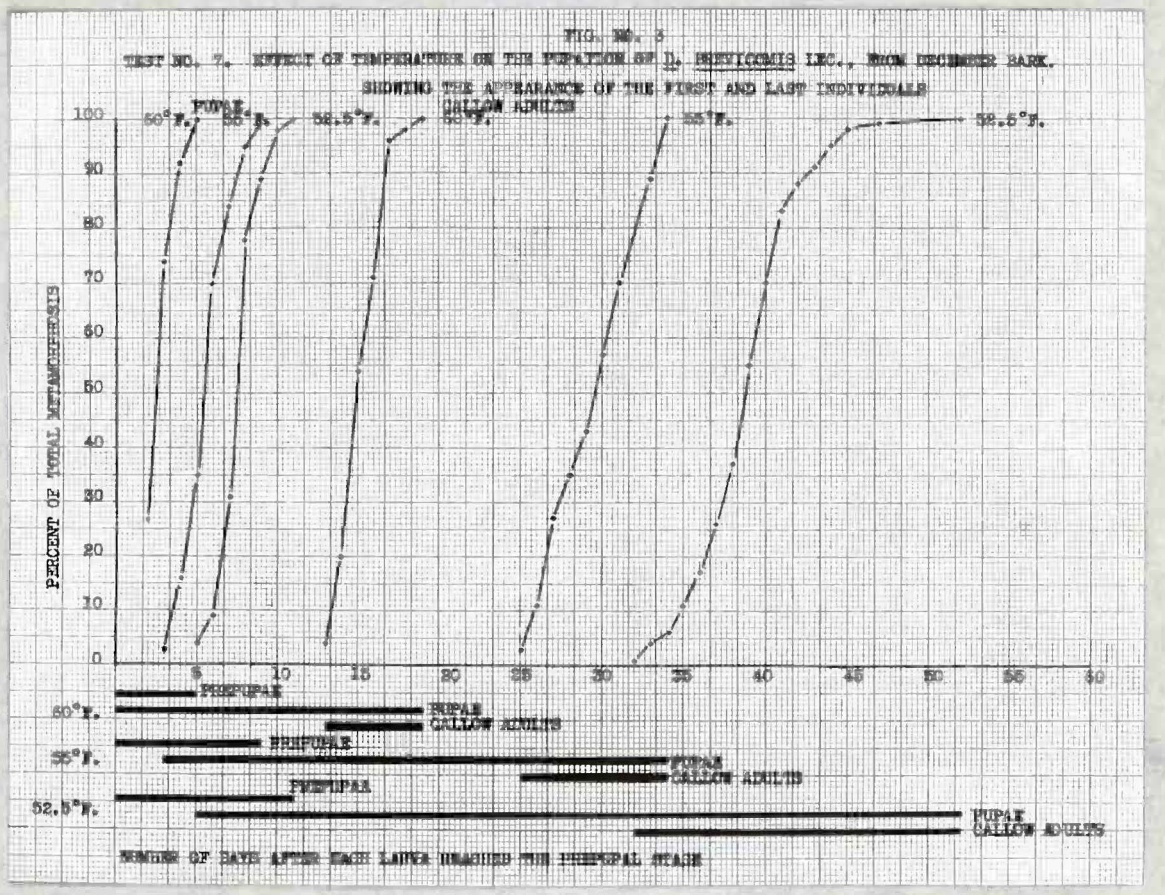


FIG. NO. 4

THE APPEARANCE OF THE FIRST PUPA AFTER THE START OF EXPOSURE TO CONSTANT TEMPERATURES, OF OVERWINTERING D. BREVICOMIS LEC. FROM BARK COLLECTED AT INTERVALS DURING THE WINTER.

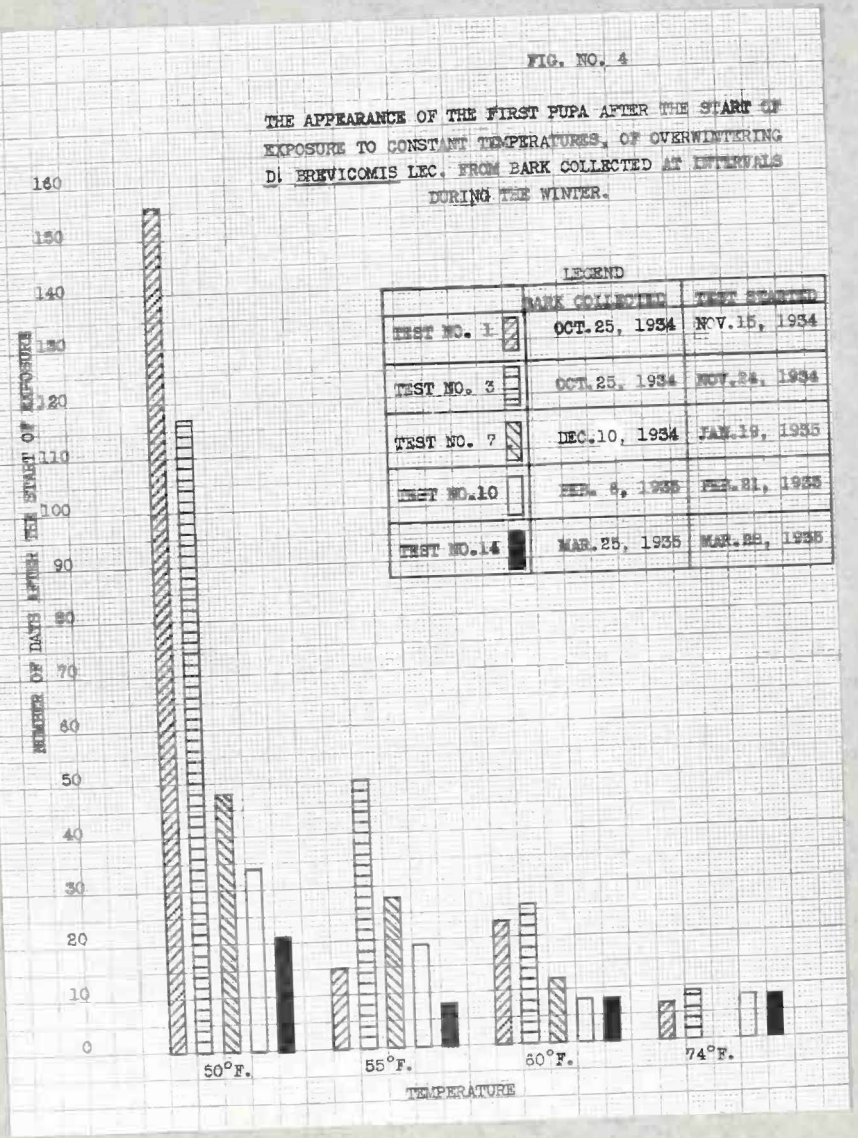


FIG.NO. 5

THE APPEARANCE OF THE FIRST CALLOW ADULT AFTER THE START OF EXPOSURE TO CONSTANT TEMPERATURES, OF OVERWINTERING D. BREVICOMIS LEC. FROM BARK COLLECTED AT INTERVALS DURING THE WINTER.

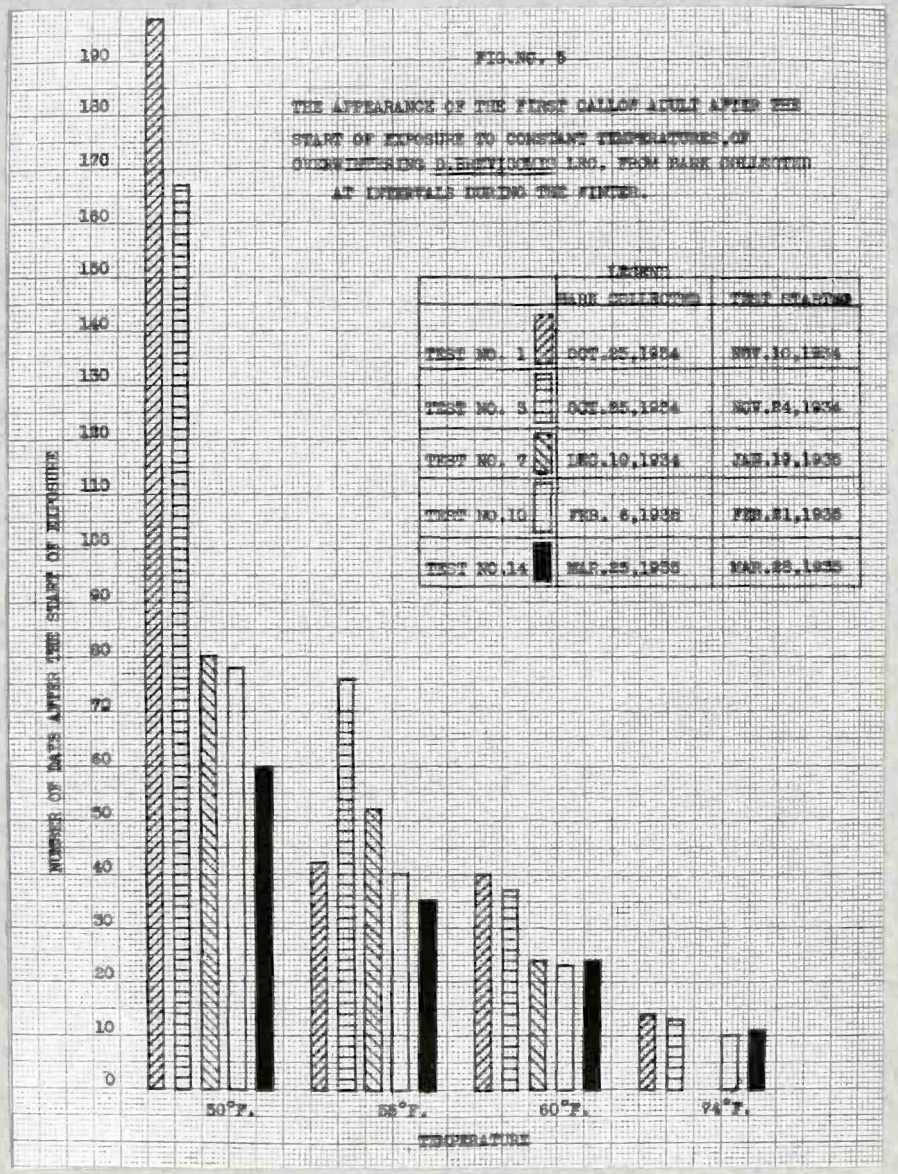


FIG. NO. 6

TEST NO. 8. THE APPEARANCE OF THE FIRST AND LAST PUPA FROM MATURE D. SEMITECTUS LEC. LARVAE STIMULATED TO THE PREPUPAL STAGE AT 74°F. THEN PLACED AT LOWER TEMPERATURES.

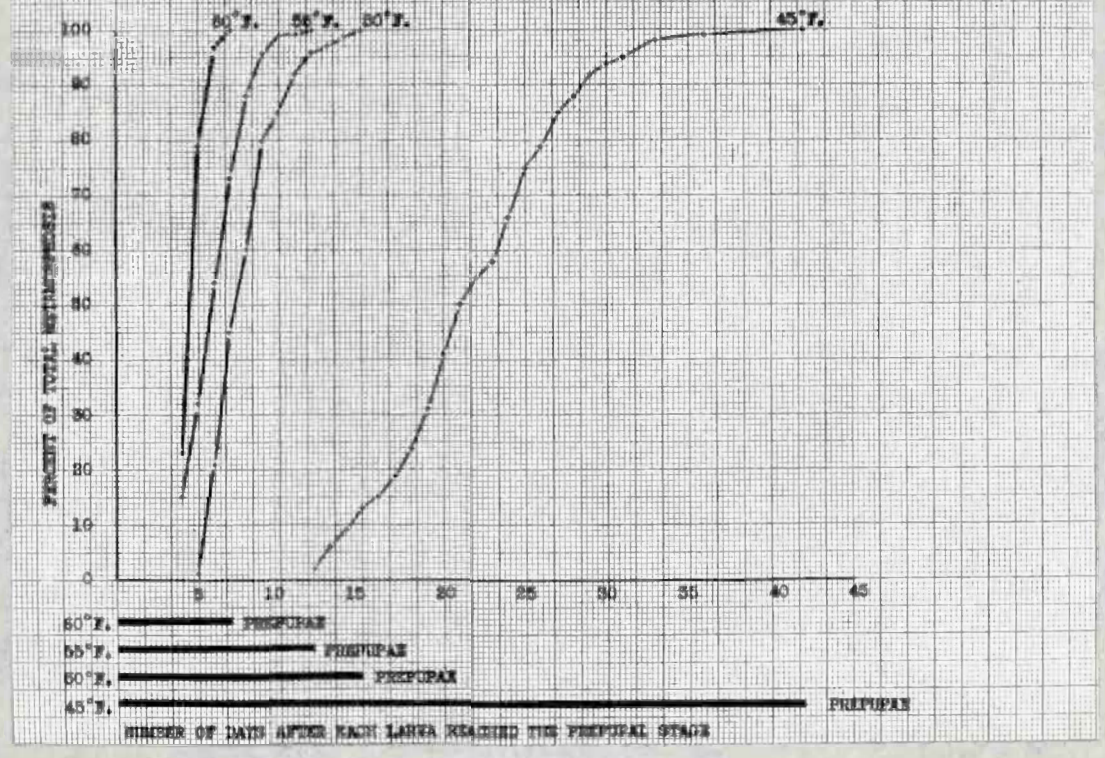


FIG. NO. 7

TEST NO. 9. THE APPEARANCE OF THE FIRST AND LAST CALLOW ADULTS FROM MATURE D. SEMITECTUS LEC. LARVAE STIMULATED TO THE PREPUPAL STAGE AT 74°F. THEN PLACED AT LOWER TEMPERATURES.

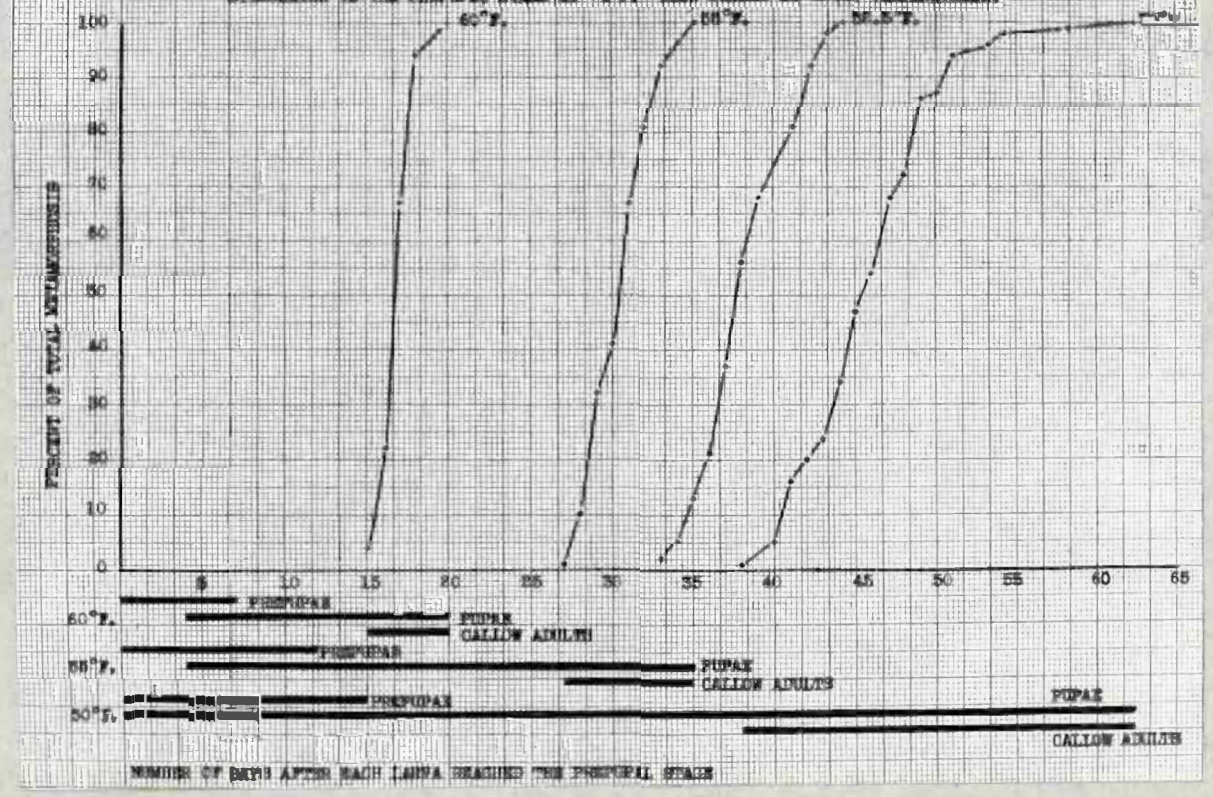


FIG. NO. 8

TEST NO. 9. THE APPEARANCE OF THE FIRST AND LAST CALLOW ADULT FROM MATURE D. SHEVIOCOMIS LEC. LARVAE STIMULATED TO THE PUPAL STAGE AT 74°F., THEN PLACED AT LOWER TEMPERATURES.

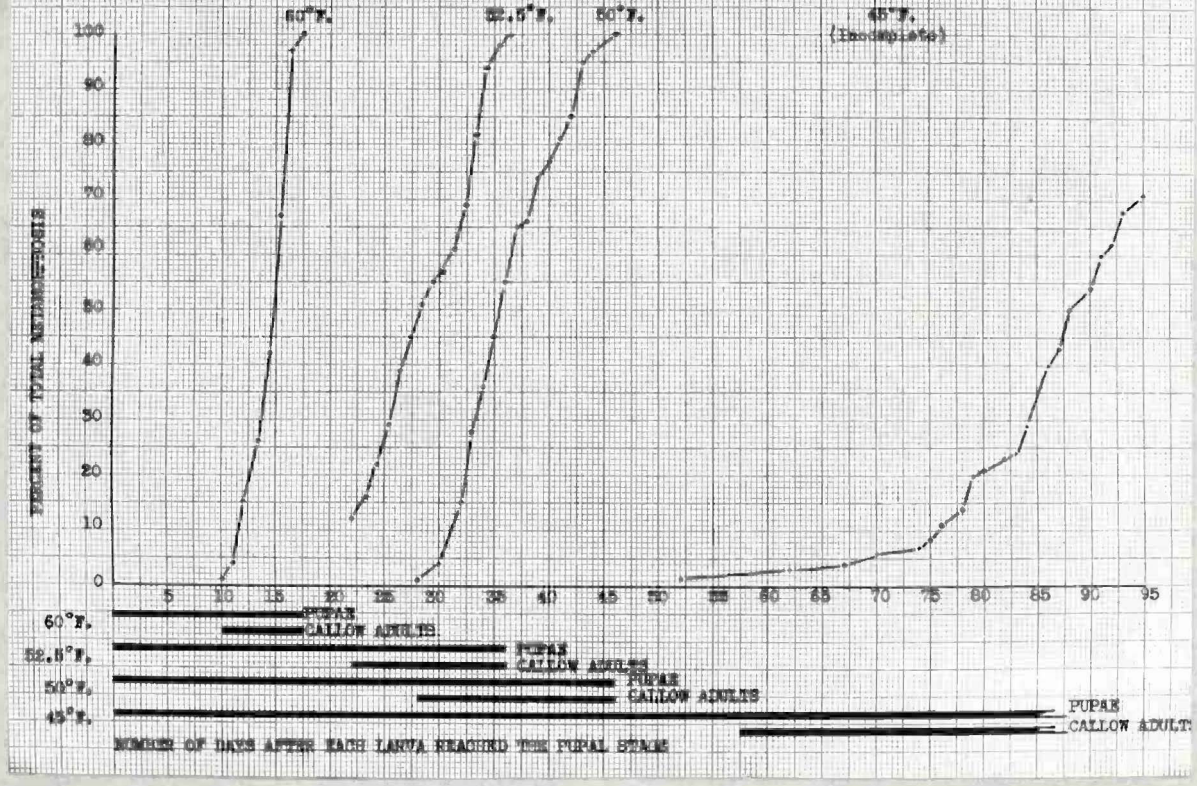


FIG. NO. 9

THE NUMBER OF DAYS AFTER THE START OF THE PREPUPAL STAGE OF OVERWINTERING D. SHEVIOCOMIS LEC. UNTIL THE FIRST CALLOW ADULT APPEARED.

